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(54) Title: THE USE OF LIPOSOMES FOR THE DELIVERY OF THERAPEUTIC AGENTS TO WOUNDS, CUTS AND ABRASIONS

(57) Abstract

Methods for delivering therapeutic agents to wounds in liposomes which preferentially bind to the wounds are disclosed. Methods for delivering therapeutic agents in liposomes to wounds on the ocular surface of the eye are included.

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THE USE OF LIPOSOMES FOR THE DELIVERY OF THERAPEUTIC AGENTS TO WOUNDS, CUTS AND ABRASIONS

Background of the Invention

This invention is directed to methods for treating wounds, cuts, and abrasions caused by injury, trauma, surgery or disease through the delivery of therapeutic healing agents to such sites. More particularly, the invention is directed to the delivery of therapeutic agents to wounds, cuts and abrasions on the cornea or ocular surface of the eye via liposome and liposome-polymer compositions which specifically adsorb or adhere to the wound, cut or abrasion.

Liposomes are sac or vesicle-like bilayer structures made of phospholipids. For an in-depth discussion of the preparation and properties of liposomes, incorporation of drugs and proteins therein and targeted drug delivery; see, G. Gregoriadis, Liposome Technology, Vols. I,II and III, CRC Press Inc., Boca Raton, Florida, 1984, the contents of which are incorporated herein by reference.

The use of liposomes as delivery vehicles for drugs or other therapeutic agents is known. Liposomes as delivery vehicles of therapeutic agents to the eye is also known; see for example, U.S. Patent No. 4,649,047 issued to R. Kaswan, March 10, 1987 disclosing the treatment of traumatic or surgical phacoanaphylactic endophthalmitis or uveitis occurring throughout the eye globe by topical administration of cyclosporin in suitable excipients, including liposomes. U.S. Patent No. 4,588,578 issued to Fountain et al., May 13, 1986 discloses the preparation of bioactive agent carrying lipid vesicles which can be applied topically for the treatment of ocular disease such as

glaucoma. Skuta et al., in American Journal of Ophthalmology, Vol.103, No.5, May, 1987 disclose the delivery of 5-fluoro-uracil in a liposome via subconjunctival injection as a method for inhibiting fibroblast proliferation following posterior sclerectomies. Skuta et al. characterize their delivery system as an advantageous, sustained release delivery system; prior methods of treatment with the free drug required frequent subconjunctival injections.

The use of liposomes for certain types of targeted drug delivery in the eye has been described previously. Norley et al., in J. Immunol., Vol.136, No. 2 (January 15, 1986) disclose the targeting of drug-loaded liposomes to Herpes Simplex virus (HSV) infected corneal cells, in vitro. The targeting moiety incorporated in the liposomes is an antiviral monoclonal antibody to glycoprotein D of HSV containing a covalently-bound fatty acyl chain. This "immunoliposome" was shown to bind specifically to HSV infected rabbit corneal cells. In another example of targeted drug delivery, intravitreally injected liposomes were used for delivery of drugs to the cells of the retinal membrane, see, Stern et al., Investigative Ophthalmology and Visual Science, Vol.28, No.5, (May, 1987). U.S. Patent No. 4,755,388 issued to Heath et al. (July 5, 1988) discloses the use of liposome-encapsulated agents such as 5-fluoropyrimidines useful in preventing scar formation by inhibiting cell contraction and cell growth in impaired tissue in the eye. Heath et al. describe a variety of covalent modifications of the liposome using ligands and receptors for targeting to specific cell surfaces or for ligand binding.

The preparation and use of bioadhesive liposomes for topical application of therapeutic agents to the ocular surface has also been described. Positively-charged liposomes and polymers are known for their nonspecific bioadhesion to surface

regions of the eye; see, Fitzgerald, et al., Journal of Pharmacy and Pharmacology, Vol.39, pp.487-490, (1987); and Shiota et al., Ocular Delivery of Vitamin A Using Positively Charged Liposomes, ARVO Annual Meeting Abstract Issue, p.159, No.22 (1987).

Figure

The sole figure illustrates adsorption of liposomes of the present invention to corneal wounds versus their relative nonadsorption to unwounded corneas.

Summary of the Invention

This invention is directed to methods for treating wounds by the delivery of therapeutic agents in liposomes to the wound site. The liposomes preferentially adsorb to wound sites and not to healthy tissue surrounding the sites.

More particularly, the invention is directed to the delivery of therapeutic agents in liposomes to wounds on the surface of the eye, such as the cornea, conjunctiva and sclera. Because the liposome carriers bioadsorb almost exclusively to the wound site on the ocular surface, the amount of therapeutic agent normally administered as a free agent can be significantly reduced. Consequently, the therapeutic index or margin of safety of a drug delivered according to the method of this invention will be improved.

Although topical ophthalmic application of drug containing liposomes is known, the prior delivery methods have been used as a means for improving the transport of therapeutic agents into and across tissue barriers of the eye, such as the cornea, conjunctiva and sclera. Targetable and bioadhesive liposomes such as those disclosed by Morley et al. (1986) and

Shiota et al. (1987) have been formulated through either covalent modification of the phospholipid or intercalation of ligand molecules into the liposome bilayer. The use of complex receptor-recognition ligands, or positively charged components have been devised as targeting agents or nonspecific bioadhesive components for tissue surfaces. However, prior methods have not recognized that liposomes will preferentially bioadsorb, with a high degree of specificity, to corneal wounds, such as cuts and abrasions. The present invention is based on the surprising discovery that liposomes adhere to wound sites but not to the surrounding healthy tissue. As used herein the term "wound" includes cuts, scrapes, abrasions or tissue damage caused by injury, trauma, surgery or disease. Thus, therapeutic agents which are known to be effective in the treatment of wounds can, according to this invention, be delivered specifically to wound sites with any nontoxic phospholipid/cholesterol liposome. In particular, the liposomes containing therapeutic agents are useful for treating wounds of the eye, particularly the cornea.

Detailed Description of the Preferred Embodiments

Liposomes which can be used according to this invention include any lipesome which bioadsorbs to a wound site, particularly a corneal wound site, and shows no toxic, inflammatory or antihealing effects. These liposomes will comprise a lipid and will typically comprise 50-90 mol% of one or more synthetic or naturally occurring phospholipids and 10-50 molar percent (mol%) cholesterol. Phospholipids which can be used in formulating liposomes for use according to the present invention include: phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine or phosphatidylserine with diacyl chains of typically 14 to 18 carbons of either the saturated or monoene variety. Other lipids, including phospholipids, which can be used in the formulation of the liposomes include, but are not

limited to: phosphatidylinositol, sphingomyelin, phosphatidic acid, cerebrosides, cardiolipin and lysophospholipids.

The liposomes can be prepared by a number of methods, including but not limited to, procedures for preparing multilamellar (MLV), small unilamellar (SUV), large unilamellar (LUV), plurilamellar (SPLV) and reverse-phase evaporation (REV) liposomes; see, G. Gregoriadis, Liposome Technology: Preparation of liposomes, Vol.1. Also see U.S. Patent No. 4,235,871, issued to Papahadjopoulos et al., incorporated herein by reference to the extent that it teaches the preparation of liposomes useful according to the present invention as described above.

It has also been found, according to the present invention, that liposomes which are relatively small, that is, 10 to 500 nanometers (nm) in diameter, preferably less than 100 nm in diameter exhibit maximal wound site coverage in comparison to the larger MLV-type liposomes of 500 to 10,000 nm in diameter.

The preferred liposome composition of the present invention comprises: phosphatidylcholine (50 mol%); the negatively charged phosphatidylglycerol (30 mol%), with both phospholipids containing acyl chains of palmitic acid in the 1-position, and oleic acid in the 2- position; and cholesterol (20 mol%). For maximum wound coverage, the preferred liposomes are 20 to 200 nm in diameter, preferably less than 100 nm. The production of liposomes of less than 100 nm is accomplished by passing MLV liposomes through a Microfluidizer^{R} (Microfluidics, Corp., Newton, Mass.) and their size will typically vary less than about 30%.

When composed of phosphatidylcholine with saturated acyl chains, the liposomes of the present invention have an aqueous liquid-crystalline phase transition of between about 35 and 42

degrees centigrade, preferably close to the human physiological temperature of 37 degrees centigrade. The liposomes of the present invention which are composed of phosphatidylcholine with monoene containing acyl chains, exhibit phase transitions well below zero degrees centigrade, and also show good bioadsorptive properties to wound sites.

This invention is not limited to only phospholipid or phospholipid/cholesterol containing liposomes, but also comprises phospholipid/cholesterol liposomes containing polymers. These liposome-polymer combinations provide for formulations with increased viscosity. Any polymer which will provide for increased viscosity around the liposomes, and is compatible with the tissues of the eye can be used. The polymer(s) are added to provide for a composition with a viscosity of between about 5 cps. and 1000 cps., preferably between about 15 cps. and 200 cps. Examples of polymers which can be used according to the methods of this invention include natural polymers such as polysaccharides (e.g. alginate, starch, modified celluloses such as hydroxypropylmethylcellulose and hydroxyethylcellulose), gelatins, albumin, casein, chitosan and collagen. In addition, synthetic polymers which can be used include polylactideglycolide copolymer, polylactide, polyhydroxyethylmethacrylate, polyesteramides, polyorthoesters, polymethacrylate, polyvinylalcohol, polyvinylpyrrolidone, polyacrylic acid, polyethyleneglycol and polymeric amino acids. The liposomes in their viscous matrix provide for longer retention of the formulation in the eye and therefore bioadsorption of the liposomes to the wound site over longer time periods. The percentage of polymer in the liposome formulation will depend on the polymer itself, but in general the concentration of polymer can range from about 0.25 - 3.0 wt.%.

Therapeutic agents which can be delivered to wound sites in liposome or liposome/polymer formulations which bioadsorb to such sites comprise all therapeutic agents useful for the treatment of wounds, cuts and abrasions caused by injury, trauma, surgery and/or disease. Such therapeutic agents will typically comprise, but are not limited to: growth factors, steroids, antioxidants, nonsteroidal antiinflammatory agents, antibiotics, immunomodulators, antiallergics and compounds which make up basement membranes, such as fibronectin and laminin. Further agents include "biological response modifiers" (i.e. agents whose mechanisms of action involve the individual's own biological response); see, Oldham, Biological Response Modifiers, Chapter 1, Torrence (Ed.) Academic Press, 1985, incorporated herein by reference.

According to the present invention, the liposomes containing the therapeutic agent or drug for the treatment of wounds are applied topically to the eye. As will be understood by those skilled in the art, the administration, sequence of administration when more than one therapeutic agent is used, and the concentrations of the therapeutic agents in the liposomes of the present invention depends on numerous factors. These factors can include: the specific therapeutic agent or agents to be used, the nature of the wound, and various clinical factors, including the extent and type of wound being treated, the medical history of the patient and symptoms associated with the wound, such as inflammation or edema, etc. Selection of the specific therapeutic agent in the liposomes, their concentration and sequence of delivery to the eye will be made by the skilled clinician guided by the foregoing description. In addition, because the liposome-type formulation bioadsorbs and/or bioadheres specifically to the wound site, the amount and frequency of therapeutic agent normally administered as the free agent can be reduced. Typically, the amount of therapeutic agent

administered in liposomes according to the present invention can be reduced by up to about 2/3 of the dose administered by other methods such as in aqueous drops, ointments or suspensions.

The following examples are directed to the binding of liposomes and liposomes comprising therapeutic agents to corneal wounds. The examples are illustrative of the preferential binding of the liposomes to wound sites, but they are in no way limiting.

Example 1

Ingredient	Concentration
Phosphatidylcholine	2.28 mg/ml
Phosphatidylglycerol	1.35 mg/ml
Cholesterol	0.46 mg/ml
Dioctadecyl-tetramethyl-indocarbocyanine (dye)	0.11 mg/ml

Preparation

Aliquots of phospholipids and cholesterol in chloroform (22.8 mg phosphatidylcholine, 13.5 mg phosphatidylglycerol, 4.6 mg cholesterol) were added to a glass container. The fluorescent dye (0.11 mg) was then added as an aliquot in methanol. The solvent was then evaporated under nitrogen. The lipid fluorescent dye film was then rehydrated with Dulbecco's phosphate buffered saline containing 0.9 mM calcium and 0.49 mM magnesium (10 ml). The pH was adjusted to 7.4 with dilute sodium hydroxide. This rehydration resulted in the spontaneous formation of multilamellar liposomes.

Freshly dissected rabbit corneas were washed in BSS Plus^{R} (Alcon Laboratories, Inc.). The corneas were placed in

plastic culture dishes and either cut or scraped medially through the epithelial surface with a scalpel blade. Immediately following the cutting or scraping, 1 µl of the fluorescent-labeled liposome formulation was applied to the corneal surface. After 0.5 min the corneas were washed 3 times with 5 ml BSS Plus^R at 37°C in a CO_2 incubator using a rotary shaker.

Each cornea was examined using fluorescence microscopy by viewing the corneas in a chamber created by two glass coverslips fitted in a machined plastic flow cell. The flow cell consists of a plastic block (11.5 X 11.5 cm X 1.2 cm) in which four 1 cm holes were bored for viewing under phase. A recessed area ~2.5 X 2.5 cm and ~2.5 mm deep was machined over and around each hole and an 18 X 18 mm coverslip adhered with silicon grease to the block within the recessed area. A thin shallow inset ledge ~0.3 mm deep was machined within the 2.5 X 2.5 cm recessed area for accommodating a top coverslip of 25 X 25 mm adhered to the ledge using silicon grease. On two opposite edges of the block, four channels, 2 per side, were machined allowing for placement of syringe needles, each tip just entering one of each of the 2.5 cm X 2.5 cm X 2.5 mm recessed chambers. Each syringe needle was held in place with a screw-washer fixed in the block. Perfusate enters the chambers through the syringe needles. In the center of the block two long channels (~8.5 cm X 1 cm and 0.5 cm deep) in fluid connection with the chambers were machined perpendicular to the channels holding the syringe needles for collection of perfusate. During examination the flow cell is fixed to the stage of the microscope with screws.

Each wounded cornea was in turn placed on an 18 X 18 mm coverslip in one chamber of the flow cell and covered with a 25 X 25 mm top coverslip. During examination the corneas were constantly perfused with BSS Plus^R and the temperature was maintained at 37°C. Video images of the fluorescent-labeled

liposomes present on the corneal surface were recorded at a magnification of 1134X. The images appeared black and white with the white regions representing those areas in which the labeled liposomes were located. The black areas in the images represented areas where no labeled liposomes were present. On observing these images, it was found that the labeled liposomes were located substantially in only the cut or scraped sites. Little to virtually no labeled liposomes adhered to the uncut or unscraped areas of any of the corneas. Nor were any labeled liposomes observed adhering to the cornea when unwounded or "normal" corneas were treated with labeled liposomes and washed according to the above described procedure.

The images were then quantitated for the area of white region (i.e. highlighted pixels) which represented the bioadsorbed liposomes. These results were expressed as cornea surface area (μm^2) as shown in the Figure with an average +/- standard error of the mean for five different images.

In the Figure it can be seen that substantially no labeled liposomes adhered to the uncut or unscraped normal cornea, whereas the labeled liposomes adhered to about 50 μm^2 in the cut corneas and 60 μm^2 in the scraped corneas. Therefore, liposomes used according to the methods of the present invention can be used to deliver wound treating agents to wounds because they preferentially adhere to wound sites and not to unwounded sites.

Example 2

The following composition can be applied topically to the eye to promote reepithelialion of a wounded cornea.

Ingredient	Concentration
Phosphatidylcholine	23 mg/ml
Phosphatidylglycerol	14 mg/ml
Cholesterol	5 mg/ml
Epidermal Growth Factor (EGF)	10 µg/ml

Preparation

For the preparation of 10 ml of composition, the phospholipids and cholesterol are weighed as dry lyophilized powders in a glass container (230 mg phosphatidylcholine, 140 mg phosphatidylglycerol, 50 mg cholesterol) followed by the addition of 1 ml t-butanol. The mixture is then solubilized by gentle mixing in a 40°C water bath, sterile filtered and then freeze dried to remove the t-butanol. The lyophilized lipid powder is then rehydrated with 100 µg of sterile EGF dissolved in Dulbecco's phosphate buffered saline containing 0.9 mM calcium and 0.49 mM magnesium (10 ml). This rehydration will result in the spontaneous formation of multilamellar liposomes containing EGF. To maximize entrapment of EGF in the liposomes, the formulation is freeze dried a second time then rehydrated with 10ml sterile deionized water before use.

Example 3

The following is an example of a liposome/polymer formulation to which therapeutic agents can be added for the treatment of corneal wounds.

Ingredient	Concentration
1-palmitoyl-2 oleoyl-phosphatidylcholine	2.3 mg/ml formulation
1-palmitoyl-2-oleoyl-phosphatidylglycerol	1.35 mg/ml formulation
cholesterol	0.46 mg/ml formulation
gelatin	25 mg/ml formulation

Preparation

The phospholipids and cholesterol are weighed as dry lyophilized powders in a glass container followed by the addition of tertiary-butanol (about 4 ml/4 mg of total lipid). Lipophilic drugs and therapeutic agents which are soluble in tertiary-butanol can be added at this point at concentrations effective for the treatment of wounds. The mixture is then completely solubilized by gentle mixing in a 40°C water bath and then freeze dried to remove the tertiary-butanol. The lyophilized powder is rehydrated with Dulbecco's phosphate buffered saline containing 0.9 mM calcium and 0.49 mM magnesium. The pH is adjusted to 7.4 with dilute sodium hydroxide. Water soluble drugs and therapeutic agents, such as growth factors, can be added at this point at concentrations effective for the treatment of wounds. Multilamellar liposomes containing drugs or therapeutic agents are formed by incubation and mixing in a rotary water bath at 40°C. For maximum entrapment of water soluble drugs and therapeutic agents, the solution can be freeze dried again and

dried again and rehydrated with water followed by further incubation and mixing in a rotary water bath at 40°C. If smaller unilamellar liposomes are desired the multilamellar liposomes can be passed through a Microfluidizer^{R} (Microfluidics, Corp.) repeatedly until the desired proportion of smaller liposomes is obtained. As will be appreciated by those skilled in the art, some liposome formulations, for example, some of those containing proteins, should not be passed through the microfluidizer because of denaturation of the protein. As a last step, powdered gelatin (Swine, skin type 1, Sigma Chemical Co., St. Louis, MO.) is added at a concentration of 25 mg/ml formulation. The mixture is then gently heated and mixed at 40°C until the gelatin is solubilized.

We Claim:

1. A method for treating ophthalmic wounds with therapeutic agents for such treatment, which comprises:

applying topically to the affected eye a composition comprising an effective amount of a therapeutic agent in liposomes which adsorb to the wounds.

2. The method of claim 1 wherein the liposomes comprise about 50-90 mol% synthetic or naturally occurring phospholipids and about 10-50 mol% cholesterol.

3. The method of claim 2 wherein the phospholipid is selected from the group consisting of:

phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylserine with acyl chains of from 14 to 18 carbons, said chains being saturated or containing one unsaturated bond.

4. The method of claim 2 wherein the liposomes comprise about 50 mol% phosphatidylcholine, about 30 mol% phosphatidylglycerol and about 20 mol% cholesterol.

5. The method of claim 4 wherein the liposomes are about 10 to 10,000 nm in diameter.

6. The method of claim 1 wherein the liposomes are about 10 to 10,000 nm in diameter.

7. The method of claim 6 wherein the liposomes are about 20 to 500 nm in diameter.

8. The method of claim 1 wherein the composition further comprises a polymer in an amount sufficient to provide the composition with a viscosity of between about 5 cps. and 1000 cps.

9. The method of claim 8 wherein in the composition the liposomes comprise about 50-90 mol% synthetic or naturally occurring phospholipids and about 10-50 mol% cholesterol and the polymer concentration is between about 0.25-3.0 wt.%.

10. The method of claim 8 wherein the polymer is selected from the group consisting of polysaccharides, gelatins, albumin, casein, chitosan, collagen, polylactide, polylactideglycolide copolymer, polyorthoesters, polymethacrylate, polyvinylalcohol, polyacrylic acid, polyvinylpyrrolidone, polyhydroxyethyl-methacrylate, polyesteramides, polyethyleneglycol and polymeric amino acids.

11. The method of claim 10 wherein the polymer is a gelatin.

12. A method for treating an ocular wound resulting from surgery to promote the healing of the wound, which comprises:

applying topically to the wound a pharmaceutical composition comprising an amount of a drug sufficient to promote healing of the wound, said drug being contained in liposomes adapted for selective binding to the wound by means of adsorption.

13. The method of claim 12 wherein the liposomes comprise about 50-90 mol% synthetic or naturally occurring phospholipids and about 10-50 mol% cholesterol.

14. The method of claim 13 wherein the phospholipid is selected from the group consisting of:

phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylserine with acyl chains of from 14 to 18 carbons, said chains being saturated or containing one unsaturated bond.

15. The method of claim 12 wherein the liposomes are about 10 to 10,000 nm in diameter.

16. The method of claim 12 wherein the liposomes are about 20 to 500 nm in diameter.

17. The method of claim 12 wherein the drug is EGF.

18. A method of selectively delivering a drug to an ophthalmic wound site, which comprises:

applying topically to the eye an ophthalmic pharmaceutical composition comprising liposomes containing a drug in an amount sufficient to achieve the desired therapeutic result, said liposomes having an affinity for binding to the ophthalmic wound site by means of adsorption.

19. The method of claim 18 wherein the liposomes comprise about 50-90 mol% synthetic or naturally occurring phospholipids and about 10-50 mol% cholesterol.

20. The method of claim 19 wherein the phospholipid is selected from the group consisting of:

phosphatidylcholine, phosphatidylglycerol, phosphatidylethanolamine and phosphatidylserine with acyl chains of from 14 to 18 carbons, said chains being saturated or containing one unsaturated bond.

21. The method of claim 19 wherein the liposomes comprise about 50 mol% phosphatidylcholine, about 30 mol% phosphatidylglycerol and about 20 mol% cholesterol.

22. The method of claim 21 wherein the liposomes are about 20 to 500 nm in diameter.

23. The method of claim 18 wherein the liposomes are about 10 to 10,000 nm in diameter.

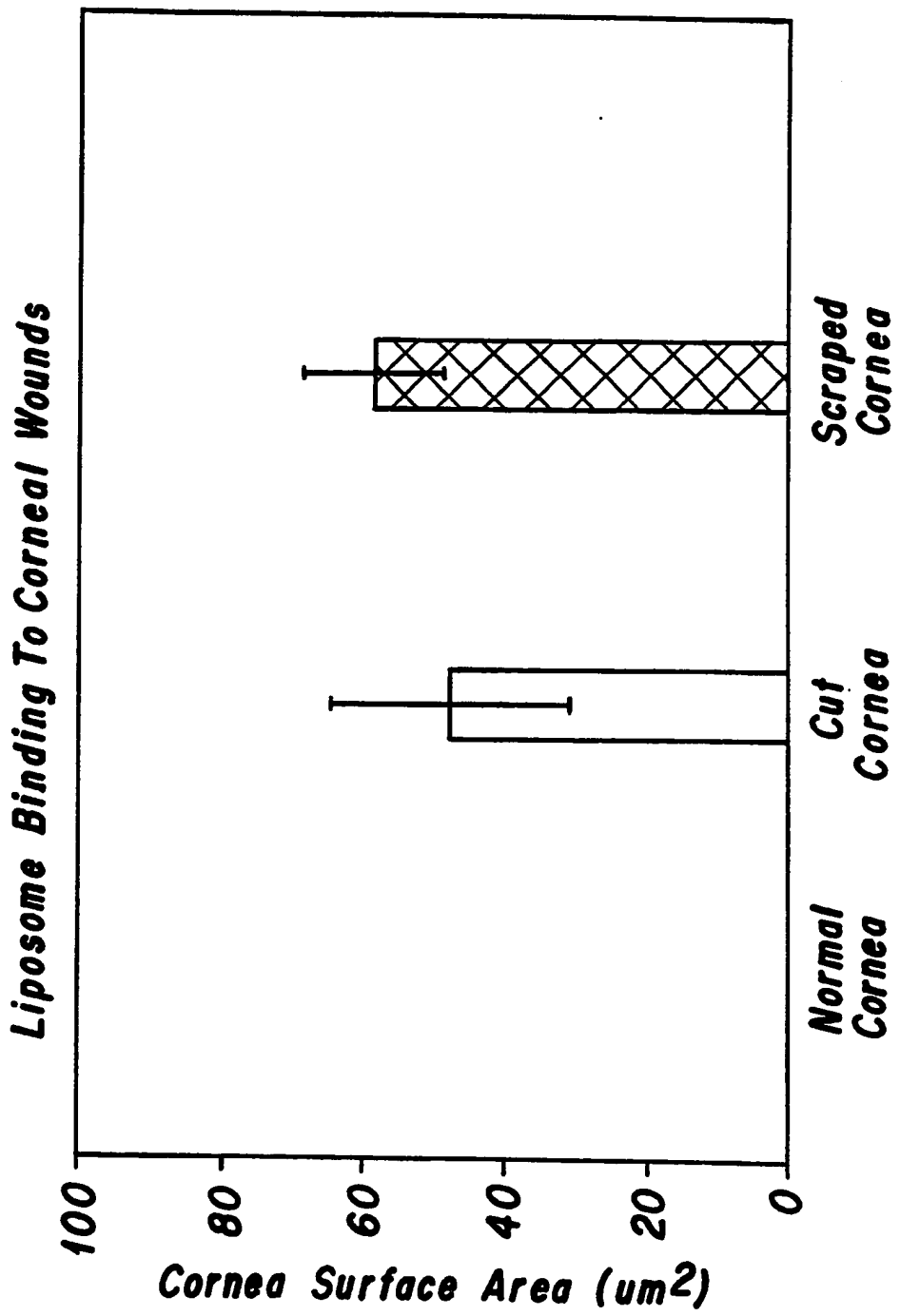
24. The method of claim 23 wherein the liposomes are about 20 to 500 nm in diameter.

25. The method of claim 18 wherein the composition further comprises a polymer in an amount sufficient to provide the composition with a viscosity of between about 5 cps. and 1000 cps.

26. The method of claim 25 wherein in the composition the liposomes comprise about 50-90 mol% synthetic or naturally occurring phospholipids and about 10-50 mol% cholesterol and the polymer concentration is between about 0.25-3.0 wt.%.

27. The method of claim 25 wherein the polymer is selected from the group consisting of polysaccharides, gelatins, albumin, casein, chitosan, collagen, polylactide, polylactideglycolide copolymer, polyorthoesters, polymethacrylate, polyvinylalcohol, polyacrylic acid, polyvinylpyrrolidone, polyhydroxyethyl-methacrylate, polyesteramides, polyethyleneglycol and polymeric amino acids.

28. The method of claim 27 wherein the polymer is a gelatin.


Liposome Binding To Corneal Wounds
Scraped Cornea
Cut Cornea
Normal Cornea
100
80
60
40
20
0
Cornea Surface Area (um2)

INTERNATIONAL SEARCH REPO

International Application No. PCT/US90/01664

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 6

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 43/00, 37/22
US CL.: 424/1.1, 450

II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System	Classification Symbols
U.S.	424/1.1, 450

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched 8

III. DOCUMENTS CONSIDERED TO BE RELEVANT 9

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
X Y, P	US,A, 4,839,175 Guo et al) 13 June 1989 See column 3, lines 3-5, 12, 48-53; column 7, lines 21-25; column 17, line 66; column 18, line 8; column 17, line 20	1-7,9-10,12-16,18-24,26-27 8,11,17,25,28
A	US,A, 4,752,425 (Martin et al) 21 June 1988 See entire document	1-28
A	US,A, 4,708,861 (Popgscu et al) 24 November 1987 See entire document	1-28
A	US,A, 4,692,454 (Mich et al) 08 September 1987 See entire document	1-28

* Special categories of cited documents: 10

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

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IV. CERTIFICATION

Date of the Actual Completion of the International Search

01 May 1990

Date of Mailing of this International Search Report

06 AUG 1990

International Searching Authority

ISA/US

Signature of Authorized Officer

Penny Prater

Form PCT/ISA/210 (second sheet) (Rev.11-87)